Rice Science, 2005, 12(3): 229－232
http://www.ricescience.org

Tissue Culture Responses from Different Explants of Rice

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Abstract: Different culture explants, including anther, young panicle, young embryo, and mature embryo, from 19 rice varieties were used for callus induction and green plantlet differentiation. The culture efficiency differed significantly among the four types of explants, and varied from genotype to genotype. Callus induction frequency presented significantly positive correlation each between anther and young panicle, anther and mature embryo, and young panicle and young embryo. Green plantlet differentiation showed no relationship between different types of explants. In addition, no relationship was found between callus induction frequency and green plantlet differentiation frequency.

Key words: rice; explant; callus induction frequency; green plantlet differentiation frequency; correlation

Explant culture has been used for crop improvement, providing a rapid method for regeneration of desired genotypes. The four explants of anther, young panicle, young embryo and mature embryo have being studied more in rice tissue culture and the culture response of them could affect breeding [1–7]. The present study was aimed to find out whether there are correlations among the culture responses of different types of explants in the same genotype. If the hypothesis were true, then it was likely to forecast the culture response of one type explant via measuring that of the other type explant, which may be a shortcut in foundamental research of rice tissue culture. It can also give the probability to research the correlation among physiological mechanisms of different explants in the same genotype.

MATERIALS AND METHODS

Rice materials

The rice materials were selected from the varieties with stable characteristics: Minghui 63, Duohui 2, CDR22, R761, R734, R130, 89-193, IR34582-19-3-2, Jiangsujing 9516, Yuanjing 2, Mawannuo, Zhendao 532, 98-116, Taihujing 2, Jiangsu 17109, Wuyujing 3, IR66159-32-6-3-4, DF213/ Minghui 63, N625/505//88/Gu H_3, Guang msF_5, Guang msF_2, Milyang 46/7071/ Minghui 63 and A6am/6323//885/Gu H3.

Received: 1 January 2005; **Accepted:** 4 June 2005
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Methods

Sampling method

Anther sampling: young panicles with pollen cell at monokaryotic stage were collected and surface sterilized with 75% ethanol, and then treated at 9–10℃ in storage bags. After a week, on a super clean worktable, the young panicles were sterilized with 75% ethanol for 30 s prior to with 0.1% $HgCl_2$ for 7–10 min, and then rinsed with sterilized water for 4–5 times. Anthers separated from the panicles with forceps were inoculated on induction media.

Young panicle sampling: healthy plants with 0.5–1.0 cm young panicles were adopted with root and upper leaves cut away. Sheaths were cleaned with 75% ethanol and placed at 4℃. After 24 h, the young panicles were sterilized with 75% ethanol for 30 s and with 0.1% $HgCl_2$ for 8–15 min and rinsed with sterilized water for 4–5 times. With sheath being moved away, the young panicles were cut into 2–3 mm long and inoculated on induction media.

Young embryo sampling: immature karyopsis (grain) were sampled 12–15 d after flowering with the lemma and palea moved. Immature seed were sterilized with 75% ethanol for 4 min and with 0.1% $HgCl_2$ for 20 min and rinsed with sterilized water for 3–4 times. Young embryos were squeezed and inoculated on induction media.

Mature embryo sampling: dehusked seeds were adopted and washed with 75% ethanol for 30 s and rinsed with sterilized water for 3 times, and then the seeds were soaked in sterilized water. After 24 h, the seeds were sterilized with 0.1% $HgCl_2$ for 30 min and thoroughly rinsed with sterilized water for 4–5 times, and then inoculated on induction media.

Media

Induction media: HE5 and common culture medium for indica rice genotypes, N6 and common culture medium for japonica rice genotypes, MS medium for young panicles and mature embryos, common culture medium for young embryos, MS medium for subculture and differentiation.

Statistical methods

The number of induced callus was recorded after different explants being cultured for two weeks except that anthers cultured for 3–4 weeks. Calli subcultured for two times were transferred to differentiation media. The number of green plantlet was recorded after three weeks.

The explants inoculation and callus transferring of different genotypes were repeated for three times. The number of anthers, young panicle segments, young embryos, mature embryos and calli inoculated at one time repeatedly was 1000, 50, 50, 50 and 50, respectively.

Callus induction frequency (CIF, %) = The number of calli / The number of inoculated explants×100%;

Green plantlet differentiation frequency (GPDF, %) = The number of green plantlet differentiation / The number of transferred calli×100%.

RESULTS

Anther culture response

The anther culture responses of the 19 genotypes indicated that most of CIF of anthers were low with an average of only 3.97%. Table 1 showed there was genotypic difference in CIF and GPDF among different rice genotypes, respectively. CIF ranged from 0 to 23.75%, and GPDF ranged from 0 to 50.85%.

Young panicle culture response

Callus induction of young panicles succeeded in 17

Table 1. Different explants culture response of different genotypes. %

Genotype	Anther		Young panicle		Young embryo		Mature embryo	
	CIF	GPDF	CIF	GPDF	CIF	GPDF	CIF	GPDF
IR66159-32-6-3-4	3.74	26.65	100	39.78	90.23	80.21	78.68	78.16
Yuanjing 2	3.32	37.82	90.77	68.42	99.17	60.20	61.75	53.48
Mawannuo	0.72	4.31	-	-	16.7	66.67	68.17	50.22
Zhendao 532	3.51	23.19	84.21	75.76	-	-	64.34	38.24
Jiangsu 17109	4.90	21.24	-	-	82.48	62.67	73.71	28.63
Jiangsujing 9516	1.42	8.33	-	-	-	-	62.07	14.96
Taihujing 2	4.33	50.85	92.68	41.88	90.15	11.11	76.38	69.81
Wuyujing 3	23.75	10.45	-	-	-	-	-	-
98-166	11.73	17.45	80.00	90.58	-	-	94.53	3.08
Minghui 63	0.00	-	79.10	63.57	78.13	25.12	68.80	10.19
IR34582-19-3-2	1.95	3.57	75.24	78.19	-	-	45.52	0.00
Duohui 2	1.30	16.67	42.20	72.41	76.60	33.33	66.85	5.92
R761	1.07	20.54	-	-	-	-	33.50	0.00
89-193	0.77	0.00	52.81	78.57	73.08	35.22	48.09	49.02
R734	0.94	5.56	58.09	50.75	33.33	48.15	65.96	30.93
GuangmsF_5	0.02	0.00	63.16	54.68	38.53	30.30	-	-
DF213/Minghui 63	11.32	19.05	70.40	61.83	74.38	39.74	-	-
GuangmsF_2	0.12	8.33	45.54	60.00	79.12	54.74	-	-
N625/505//88/Gu H3	0.53	48.25	86.25	45.21	56.03	88.57	-	-
Milyang 46/7071/ Minghui 63	-	-	6.48	42.10	-	-	-	-
A6am/6323//885/Gu H3	-	-	56.16	67.81	-	-	-	-
R130	-	-	88.80	73.17	81.01	66.67	45.93	58.93
CDR22	-	-	-	-	-	-	49.67	28.14
Average	3.97 b	17.90 c	69.17 a	62.63 a	69.21 a	50.13 a	62.75 a	32.48 b
Standard deviation	5.88	14.82	22.06	15.02	24.06	22.13	15.25	25.51

CIF, Callus induction frequency; GPDF, Green plantlet differentiation frequency. F_{CIF}=54.655; F_{GPDF}=16.059.

different genotypes. Glume began to enlarge after being inoculated on induction media for 5–10 d. Calli formed after 15–20 d, and then came into being embryonic calli. The culture response of different genotypes was listed in Table 1. Most of the culture responses of young panicle were satisfying. Average CIF reached 69.17% and GPDF reached 62.63%.

Young embryo culture response

Young embryos of 14 different genotypes were inoculated. Callus induction was easier for young embryos than for the other three explants. Coleoptile emergence and calli formed firstly in three days. The majority of calli were induced in seven days, callus was primrose and compact. There was genotypic difference in young embryos culture. For example, R761 had been inoculated for two times and with no calli induced successfully. The reasons may lie on twofold: one was that the physiological characteristic of explants had difference in various inoculation time; the other was that man-made error in medium elements. The culture response of different genotypes was listed in Table 1. The CIF of young embryos ranged from 16.70% to 99.17%, with an average of 69.21%; GPDF ranged from 0 to 88.57%, with an average of 50.13%.

Mature embryo culture response

The treatment method of mature embryos in this experiment made the contaminated number less than the method that mature embryos were inoculated directly after being sterilized generally. Calli emerged almost synchronizing and more scutellum calli formed in 10 d, a few white coleoptile calli formed in 10–15 d with a little hard texture. Some mature embryos calli would be browning to death, which was attributed to the location of calli on media taken on orientation, or calli received mechanical damage in transferring process. There was genotypic difference in mature embryos culture (Table 1). CIF ranged from 33.50% to 94.53%, with an average of 62.75%; GPDF ranged from 0 to 78.16% with an average of 32.48%.

Comparison of different explants culture response

The results of variance analysis were showed in Table 1. The CIF of young panicles, young embryos and mature embryos were significantly higher than that of anthers, respectively. There was no significant difference between young panicles and young embryos; However, the GPDF of young panicles and young embryos were higher than that of mature embryos and anthers, and the GPDF of mature embryos were higher than that of anthers.

Correlation analyses of culture response of different explants from the same genotype

Correlation analyses of the culture responses by different explants were listed in Table 2. There were significantly positive correlation among CIF of anthers, young panicles and mature embryos, so were those among young panicle and young embryos. There was no correlation among the GPDF of different explants. There was no correlation between CIF and GPDF of the four explants (anther, young panicle, young embryo and mature embryo), indicating that high CIF did not imply high GPDF.

DISCUSSION

Culture responses of experimental plant materials determine its tissue culture application; genotypes and types of explants were the major factors on culture response. In this experiment, there was significant difference among different genotypes of the same explant and among different explants of the same genotype. The variance of different explants and

Table 2. Correlation coefficient of callus induction frequency and green plantlet differentiation of different explants. %

Characters		CIF				GPDF		
		Anther	Young panicle	Young embryo	Mature embryo	Anther	Young panicle	Young embryo
CIF	Young panicle	0.537*					-0.300	
	Young embryo	0.376	0.678**					-0.140
	Mature embryo	0.501*	0.379	0.134				
GPDF	Anther	-0.036						
	Young panicle		-0.300			-0.136		
	Young embryo			-0.140		-0.052	0.017	
	Mature embryo				0.097	-0.174	0.174	-0.447

*,** Significant at 0.05 and 0.01 levels, respectively.

genotypes resulted from the different physiological characteristics and developmental stages, which led to the different concentrations and ratios of endogenous hormones [8].

Rice tissue culture response comprises of CIF and GPDF, they affects rice plant regeneration. In this experimental result, there were significantly positive correlation among anthers, young panicles and mature embryos, so were those among young panicles and young embryos. All of these indicated that there was certain correlation between different explants of the same genotype. That is to say, we could estimate CIF of one along. There was no correlation among GPDF of different explants, which showed that GPDF was not only related to characteristic. The good calli quality was the key factor of GPDF increasing.

In this experiment, there was no correlation between CIF and GPDF of the four explants. It accorded with the result of Gao et al [9].

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